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ALTERED BILE TRANSPORTER EXPRESSION AND CHOLESTEROL METABOLISM IN CHILDREN WITH CHOLESTEROL AND PIGMENT GALLSTONES

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Abstract

Objectives. We elucidated pathophysiology of pediatric gallstone disease by assessing liver expression of bile transporters in relation to bile acids and surrogates of cholesterol absorption and synthesis in serum and gallstones.

Methods. RNA expression of canalicular bile transporters in liver biopsies from 32 pediatric gallstone patients and from six liver donors (controls) was measured by qRT-PCR. Concentrations of cholesterol and precursors, plant sterols and bile acids in gallstones, and in serum of the patients and 82 healthy children were measured. Primary outcomes were the difference in RNA expressions and serum sterol profiles between patients and controls.

Results. Cholesterol stones (CS; n=15) contained cholesterol >42% and pigment stones (PS; n=17) <9% of weight. CS-patients had markedly lower serum plant sterols (absorption) and higher cholesterol precursors (synthesis) than PS-patients or healthy controls. CS contained several times more cholesterol precursors and less plant sterols relative to cholesterol than PS, which were enriched by primary bile acids (12-5.2 fold, $p<0.001$). Liver RNA expression of *ABCG5/G8* was similarly increased 2.5-1.8 fold ($p<0.002$) in CS and PS-patients, while PS-patients had higher *ABCB11* expression ($p<0.05$). In PS bile acid concentration correlated with gallstone plant sterols ($R^2 = 0.83$, $p<0.0001$), and *ABCG5* expression with *ACBC11* expression ($R2 = 0.27$, $p = 0.03$).

Conclusions. In CS, upregulation of *ABCG5/G8* expression associates with low absorption and high gallstone content of cholesterol. In PS, activation of bile acid transport by *ACBC11* interconnects with hepatic upregulation of ABCG5/G8 enriching PS with bile acids and plant sterols.

Keywords: Plant sterols, bile acids, RNA expression

What is new?

- Gallstone formation in children is related with increased hepatic expression of hepatocyte sterol transporter ABCG5/G8
- Cholesterol stones (CS) are associated with decreased absorption and increased synthesis and biliary secretion of cholesterol and its precursors
- In pigment gallstones (PS) activation of bile acid transport by *ABC11* interconnects with hepatic upregulation of ABCG5/G8 enriching PS with bile acids and plant sterols

What is known?

- The incidence of pediatric gallstone disease is increasing
- In children PS are as common as CS
- Gallstone formation is related with altered cholesterol metabolism

Introduction

Pediatric gallstone disease and cholecystectomy rate are increasing in western world for unclear reasons (1). In adults, cholesterol stones (CS) are by far the most common gallstone type known to associate with biliary hypersecretion of cholesterol, obesity and features of metabolic syndrome such as decreased insulin sensitivity and liver steatosis (2,3,4). The risk factors for pediatric gallstones are less clearly defined (5,6). Contrary to adults, pigment stones (PS) form a significant proportion of gallstones in children (7,8). PS are composed mostly of calcium bilirubinate and often associate with variable underlying conditions such as hemolysis, portal hypertension, inflammatory bowel disease and ileal dysfunction (6, 8, 9, 10, 11).

Alterations in the cholesterol metabolism and supersaturation of bile with cholesterol over phospholipids and bile acids, are closely related with development of CS (4, 12). Serum plant sterols are surrogates for intestinal cholesterol absorption (13), whereas serum cholesterol precursors mirror the whole body cholesterol synthesis (13, 14, 15). Studies in both adults and children have demonstrated that CS associate with decreased intestinal cholesterol absorption as measured by low serum plant sterols (7, 8, 12), while enhanced biliary secretion in patients with CS is reflected by the accumulation of cholesterol and plant sterols into bile (12) and gallstones (8). By definition, PS have considerably lower cholesterol concentration, and weaker connections to cholesterol metabolism than CS (5, 8). Despite normal intestinal cholesterol absorption, children with PS showed increased serum surrogates of cholesterol synthesis and high gallstone bile acid content (7), which could be attributable to increased biliary secretion of bile acids by canalicular bile acid transporter ABCB11.

ATP-binding cassette (ABC) transporters ABCG5 and ABCG8 not only transport absorbed cholesterol and plant sterols from enterocytes back to the intestinal lumen, but also from hepatocytes to the bile canaliculus. Accordingly, enhanced function of ABCG5/8 in the liver and

the intestine should lead to increased secretion of cholesterol and plant sterols into bile as well as back into the intestinal lumen and decreased intestinal absorption. Indeed, a recent study showed that a gain-of-function genetic variant D19H of *ABCG8* predisposes to gallstones in humans (16), and a meta-analysis of genome-wide association studies identified four gene loci for *ABCG8* as susceptibility loci for gallstone disease (17,18). In adult gallstone patients, up-regulated hepatic expression of *ABCG5/8* has been associated with cholesterol supersaturation of bile (19). Whether similar up-regulation of *ABCG5/8* is actually present in pediatric gallstone patients and whether there are differences in bile transporter expression between CS and PS remains unknown.

In the present comparative cross-sectional study, we aimed to explore the roles of canalicular sterol, phospholipid, bile acid and bilirubin transporters in pathophysiology of pediatric gallstone disease. For this, we measured hepatic RNA expression of the transporters and their nuclear receptor upstream regulators liver x receptor (*LXR*) and farnesoid X receptor (*FXR*), and assessed their relation to serum and gallstone cholesterol, cholesterol precursors, plant sterols and bile acids. We hypothesized that *ABCG5/8* expression is upregulated in patients with CS, whereas bile acid secretion would associate with development of PS.

Patients and Methods

Ethics

The institutional ethical committee approved the study (&27/2010, 434/13/03/2008). An informed consent was obtained from patients and controls and/or their caregivers before any experimental procedures.

Patients and controls

From 1994 to 2014 we performed cholecystectomy for 80 pediatric patients with cholecystolithiasis (1994-2003 n=21, 2004-2014 n=59). Thirty-two consecutive pediatric patients with median age 13 (10-16) years, who underwent cholecystectomy for symptomatic gallstones from March 2009 to January 2014 were enrolled. Control liver specimens were obtained from transplant donor livers (n=6) with median age 23 (range 11- 42) years (p=0.004 vs patients) and control serum samples were withdrawn from generally healthy day-surgery patients (n=82) with median age 8.9 (4.1-16) years (p=0.05 vs patients) without known gastrointestinal, hepatobiliary, endocrine or lipid metabolism disorders. (Suppl Figure 1, Supplemental Digital Content, http://links.lww.com/MPG/B642)

Study procedures

Fasting serum samples and liver biopsies were obtained at the time of cholecystectomy. Gallstones were retrieved from the removed gallbladder (n = 31), or during endoscopic sphincterotomy (n = 1). During cholecystectomy two 23 mm core needle biopsies were obtained under laparoscopic control from the right liver lobe.

Serum total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol and serum triglycerides were analyzed enzymatically. Our hospital laboratory analyzed routine liver biochemistries, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AFOS) glutamyl transferase (GT), bilirubin, conjugated bilirubin, bile acids and prealbumin. Classification of the gallstones into CS and PS was performed as described earlier (2, 10).

Analyses of non-cholesterol sterols in serum and gallstones

Serum and liver samples were snap frozen and stored at -20°C, and gallstones were dried and stored in room temperature until analyzed. Serum and gallstone cholesterol, cholesterol precursors (cholestenol, lathosterol and desmosterol), cholestanol and plant sterols (campesterol, sitosterol, stigmasterol and avenasterol) and gallstone bile acids (chenodeoxyocholic, lithocholic, ursodeoxycholic, cholic and deoxycholic acid) were measured as trimethylsilylethers from nonsaponifiable material by capillary gas-liquid chromatography with flame ionization detection and using 50-m nonpolar capillary columns (Ultra 1 and Ultra 2, Agilent Technologies, Palo Alto, California), with 5α-cholestane as an internal standard as described in detail previously (20). The non-cholesterol sterols were expressed as ratio to the cholesterol concentration of the same gas-liquid chromatography run (100 x µg/mg of cholesterol), and bile acids as µg/100mg of stone. We calculated the ratios lathosterol/sitosterol and lathosterol/cholestanol, for biomarkers of whole-body cholesterol metabolism (13, 21) and campesterol/cholestanol for a biomarker of dietary plant sterol intake. (22)

Liver and gallbladder histology

Liver and gallbladder specimens were fixed in formalin, embedded in paraffin, sliced, and stained with hematoxylin and eosin, reticulin, herovici, Pearl´s iron, Periodic acid-Schiff´s, and cytokeratin-7.

RNA isolation and expression analysis

Liver tissue specimens were embedded in RNAlater-solution (Ambion, Life technologies, Thermo Fisher Scientific Inc., Waltham, MA, USA) and frozen until analyzed. RNA was extracted with the RNAeasy Mini Kit (QIAGEN, Frederick, Maryland, USA) and RNA integrity was assessed spectrophotometrically. RNA expression (*LXR, FXR, ABCG5, ABCG8, ABCB4,*

ABCB11 and ABCC2) was analyzed in triplicate by quantitative real-time polymerase chain reaction using PCR Array (QIAGEN SA Biosciences, Frederick, Maryland, USA) on an ABI 7700 Sequence Detection System (Perkin-Elmer Life Sciences, Boston, MA, USA) according to the manufacturer's instructions. Quantification of target gene mRNA expression was performed using the ΔΔCt method and expressed as fold change after normalization to housekeeping genes and relative to control subjects.

Statistical analyses

In order to ascertain adequate size of the study groups 2-sided power analysis calculation was performed for statistical power of 0.80, p=0.05. The study groups were of adequate size for the comparison of stone concentrations of sterols and bile acids between CS and PS and for the comparison of the most important serum sterol concentrations between patients and controls. In the comparison of RNA expressions we assumed that 1.5 vs 1.0 fold difference (SD 0.3 fold) was significant. Power calculations indicated adequate size of the study cohorts, small cohort size (patients n=32, controls n=6) reduced reliability of the calculations.

Unless otherwise stated, data are expressed as medians (interquartile range) or means (95% confidence interval). Multiple comparisons between groups were performed with Kruskal Wallis Test. If the group effect was significant in multiple comparisons, post hoc pairwise comparisons were performed with Mann Whitney U test. Fisher's exact was used to compare frequencies of categorical variables, and Spearman rank correlation test to assess linear relationships between variables. A p-value less than 0.05 was considered statistically significant.

Results

Patients and controls

The gender distribution was comparable between patients (females 19/32, 59%) and controls (females 40/88, 45%, p=0.22). Median Iso-BMI was 23 (19-27) kg/m^2 in patients and 23 (20-25) kg/m^2 in controls (p=0.89). Liver biochemistry values were in normal range in patients, serum controls (n=82) and in six liver donors. Donor liver biopsies showed normal histology (n=5) or mild steatosis (n=1). None of patients or controls had medications affecting cholesterol metabolism (e.g statins) or composition of bile (e.g ursodeoxycholic acid).

Baseline characteristics of patients with CS and PS are outlined in Table 1. Iso-BMI and serum total cholesterol and LDL cholesterol levels were significantly higher in CS-patients when compared to PS-patients The frequency of histologic alterations in liver were comparable in both groups.

No surgical complications occurred during cholecystectomy and liver biopsy. Before cholecystectomy four patients (CS n=2, PS n=2) underwent endoscopic sphincterotomy for removal of common bile duct stones. All patients were discharged by the third postoperative day. At outpatient clinic 1-2 months postoperatively all had normal abdominal ultrasonography and plasma liver biochemistry values (data not shown).

Predisposing conditions for gallstones

Twenty-one (66%) patients had underlying conditions that potentially predispose to gallstone disease (16-18). Overall, predisposing conditions occurred equally often in CS patients (10/15) and PS patients (11/17; p = 0.99) and in male (8/13) and female (13/19; p = 0.72) gallstones patients. Apart from obesity, 5/15 (33%) CS-patients and 11/17 (65%) PS-patients had any other predisposing conditions (p=0.16), which are detailed in a separate table (Suppl Table 1, Supplemental Digital Content, http://links.lww.com/MPG/B642). Three PS-patients had an associated intestinal disorder including Crohn's disease, necrotizing enterocolitis and gastroschisis.

Gallstone cholesterol and non-cholesterol sterols

Median stone weight of CS and PS was 57 mg (26-136) and 22 mg (14-41), respectively (p = 0.04). Cholesterol concentration (% of weight) was 85% (76-89) in CS and 0.6% (range 0.3-1.8) in PS (p = 0.0003). Total plant sterol concentrations (% of weight) were 14% (8.6-15) in CS and 0.3% (0.1-0.6) in PS (p<0.001). All PS were classified as black PS.

Cholesterol precursor (cholestenol. lathosterol and desmosterol), cholestanol and plant sterol (campesterol, sitosterol, stigmasterol and avenasterol) ratios to cholesterol are given in. Cholesterol precursors to cholesterol ratios were markedly lower and those of plant sterols sitosterol, avenasterol and stigmasterol markedly higher in PS compared to CS, while cholestanol ratios were similar between the groups. (Suppl Table 2, Supplemental Digital Content, http://links.lww.com/MPG/B642).

Gallstone bile acids

Total bile acid concentration (% of weight) was 1.6% (0.4-3.0) in CS and 9.5% (3.6-11) in PS, (p=0.01) Absolute total concentration of bile acids was ~7.0 times higher in PS than in CS (Table 3). The increase was most striking for the primary bile acids, chenodeoxycholic acid and cholic acid. PS chenodeoxycholic acid concentration was ~12 fold higher when compared to CS (Suppl Table 3, Supplemental Digital Content, http://links.lww.com/MPG/B642). In PS-patients gallstone bile acid concentration correlated with total plant sterols to cholesterol ratio (R2 = 0.83, p<0.0001) and cholesterol concentration (R2=0.48, p=0.003).

Serum cholesterol precursors and plant sterols

Results of serum cholesterol precursors, cholestanol, and plant sterols are shown in Table 2. Compared to both control subjects and PS-patients, patients with CS had significantly higher serum cholestenol and lathosterol ratios to cholesterol and significantly lower ratios of plant

sterols and cholestanol. Lathosterol/sitosterol and lathosterol/cholestanol ratios were ~3-5 times higher in CS than in PS or controls ($p<0.001$). Only CS-patients showed decreased campesterol/cholestanol ratio when compared to controls.

In patients with CS there was a positive correlation between serum and gallstone cholesterol precursors and plant sterols ratios to cholesterol ($R2 = 0.50$, $p = 0.005$ and $R2 = 0.97$, $p < 0.0001$, respectively). In patients with PS no such correlations were observed ($R2 = 0.15$, $p = 0.16$) and $R2 = 0.12$, $p = 0.19$) suggesting that cholesterol homeostasis was perturbed.

RNA expression

The results of RNA expression analyses are outlined in Figures 1 and 2. Among all gallstone patients RNA expression of *LXR* was ~1.4 times higher than in controls ($p=0.06$), but the difference reached statistical significance only for PS-patients ($p = 0.048$) (Suppl Figure 1, Supplemental Digital Content, http://links.lww.com/MPG/B642). RNA expression of *ABCG5* was ~2.5 times ($p = 0.001$) and that of *ABCG8* ~1.8 times higher in all gallstone patients than in controls ($p = 0.01$), and these increases were similar between CS- and PS-patients

Liver RNA expression of bile acid transporter *ABCB11* was ~1.5 times higher in PS-patients compared to CS-patients ($p = 0.04$), although the difference in relation controls remained statistically insignificant (Supplemental Digital Content, http://links.lww.com/MPG/B642). In patients with PS, *ABCG5*expression correlated positively with expression of *ACBC11* ($R2 = 0.27$, $p = 0.03$). No meaningful differences in RNA expression of *FXR*, bilirubin transporter *ABCC2* and phospholipid transporter *ABCB4* were observed between groups (data not shown). Liver RNA expression of any of the studied bile transporters did not correlate with age, gender, liver steatosis, plasma liver biochemistry values or sterol levels in serum and gallstones either in patients or controls.

Discussion

The main new findings of the present study showed that liver RNA expression of sterol transporter *ABCG5/G8* is similarly increased in children with CS and PS. In addition, *ABCG5* expression associated with activated expression of bile acid transporter *ABCB11* and markedly higher gallstone primary bile acid concentration in PS when compared to CS. Finally, liver RNA expression of bilirubin transporter *ABCC2*, phospholipid transporter *ABCB4* were unaltered in both gallstone subgroups. Our measurements of serum surrogate markers of cholesterol absorption indicated that CS-patients had low intestinal absorption of cholesterol, while PS-patients exhibited similar cholesterol absorption to control subjects. Overall synthesis of these findings implicate that upregulation of ABCG5/G8 is the primary event in formation of CS, resulting in both decreased intestinal absorption and increased biliary secretion of cholesterol leading to formation of CS composed mostly of cholesterol. In PS, activation of *ABC11* expression and biliary secretion of bile acids may trigger selective upregulation of ABCG5/G8 in the liver (23), enriching PS with primary bile acids and plant sterols relative to cholesterol.

Low intestinal cholesterol absorption as assessed by serum plant sterols associates with formation of CS in both children and adults (8, 12). Individuals who develop gallstones in adulthood, display the metabolic trait of low cholesterol absorption already decades earlier in childhood, suggesting that low cholesterol absorption is the primary, possibly genetic attribute, which leads to increased cholesterol synthesis as in our CS-patients (8, 24). Indeed, polymorphism of ABCG8 gene associates with the metabolic trait of low surrogate sterol markers of cholesterol absorption in childhood, although presence of the risk allele 19H does not explain formation of gallstones alone (12, 25). Our findings extend previous observations by showing that of the various canalicular bile transporters studied, only expression of sterol transporter *ABCG5/G8* was upregulated in children with CS. Notably, phospholipid transporter *ABCB4* expression was unaltered in accordance with an earlier study showing an association

between ABCB4 mutations and low phospholipid cholelithiasis syndrome but not with idiopathic pediatric CS (26). Although we did not measure sterol transporter RNA expression in enterocytes, the simultaneously low surrogate markers of cholesterol absorption indicates enhanced ABCG5/G8 function also in the intestine (27). These findings support the primary role of activated ABCG5/G8 in driving biliary cholesterol hypersecretion in the pathogenesis of pediatric CS.

PS are thought to arise from biliary hypersecretion of bilirubin with contributions from the enterohepatic circulation of bile acids (11, 28). Malabsorbed bile acids may facilitate intestinal absorption and recirculation of unconjugated bilirubin, while high biliary bile acid concentration at the expense of cholesterol and phospholipids may cause detergent injury to gallbladder mucosa and thereby facilitate gallstone formation (11). Cholestanol is a sensitive biomarker of decreased bile flow and elevated serum cholestanol concentration in PS patients suggests an association between bile stasis and formation of PS (29, 30). While three of our PS-patients had an underlying hemolytic disease, we did not observe elevated mean expression of canalicular bilirubin transporter *ABCC2* and all had normal serum bilirubin at the time of the study. Instead, expression of sterol transporter *ABCG5/8* and its upstream nuclear receptor regulator *LXR* were significantly increased in patients with PS. Moreover, we noted upregulation of canalicular bile acid transporter *ACBC11* and 12 to 5 fold higher gallstone primary bile acid concentration in PS compared to CS. Similarly to our previous study, the mostly enriched bile acid in PS was a primary bile acid chenodeoxycholic acid (8). Moreover, we found positive correlations between liver bile acid transporter *ACBC11* and sterol transporter *ABCG5* expression, and between gallstone bile acids and plant sterols, implicating that biliary secretion of bile acids and plant sterols were linked with each other in patients with PS. Interestingly, bile acids, and especially chenodeoxycholic acid, increase *ABCG5* expression in cultured human hepatocytes (23). Together these data indicate that bile acids may have a dual role in formation of PS by causing

detergent mucosal injury in increased gallbladder concentrations, and by activating *ABCG5* expression and biliary secretion of sterols. In patients with PS, relatively more plant sterols accumulated into gallstones over cholesterol putatively due to its lower hepatic clearance rate and limited availability as reflected by the low serum cholesterol concentration (31). Unexpectedly, the surrogate sterol markers of cholesterol synthesis in patients with PS were not increased above control levels as in our previous study including 35% of PS-patients with underlying intestinal absorption defects (12). The normal median level of cholesterol synthesis surrogates indicates that most PS-patients in the current study had intact enterohepatic circulation of bile acids in addition to normal cholesterol absorption as assessed by serum sterol surrogates. Thus, activation of biliary bile acid secretion may also occur in the absence of significant bile acid malabsorption and still contribute to PS formation. Indeed, only three (18%) of our 17 PS-patients had an underlying intestinal disease predisposing to bile acid malabsorption.

The main limitations of this study includes small number of patients and control donor liver biopsies for RNA expression measurements. In addition, different control subject cohorts were used for measurements of RNA expression and serum sterol surrogates, and the age of patients and controls were not statistically comparable, although both the patients and serum controls belonged to pediatric population. Decreased serum campesterol / cholestanol ratio, in general suggesting low dietary intake of plant sterols, is conceivably not a valid biomarker in CS - patients because campesterol and cholestanol levels are low in these patients because of decreased cholesterol absorption efficiency. Finally, we did not include Niemann-Pick C1- like 1 transporter in our expression studies, although it seems to have a minor role in the pathophysiology of gallstone disease (25). However, by combining expression of liver bile transporters with surrogates of cholesterol metabolism and bile acid profile in gallstones, this study provides novel information on pathogenesis of pediatric gallstone disease.

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Figure legends

Figure 1. Liver RNA expression among all gallstone patients (ALL, n=32), patients with cholesterol stones (CS, n=15), patients with pigment stones (PS, n=17) and controls (n=6) for (a) *LXR*, (b) *ABCG5*, and (c) *ABCG8*. Data are fold changes (mean with 95% confidence interval) in relation to controls. *p < 0.05 vs controls **p<0.05 vs PS (Mann-Whitney U test).

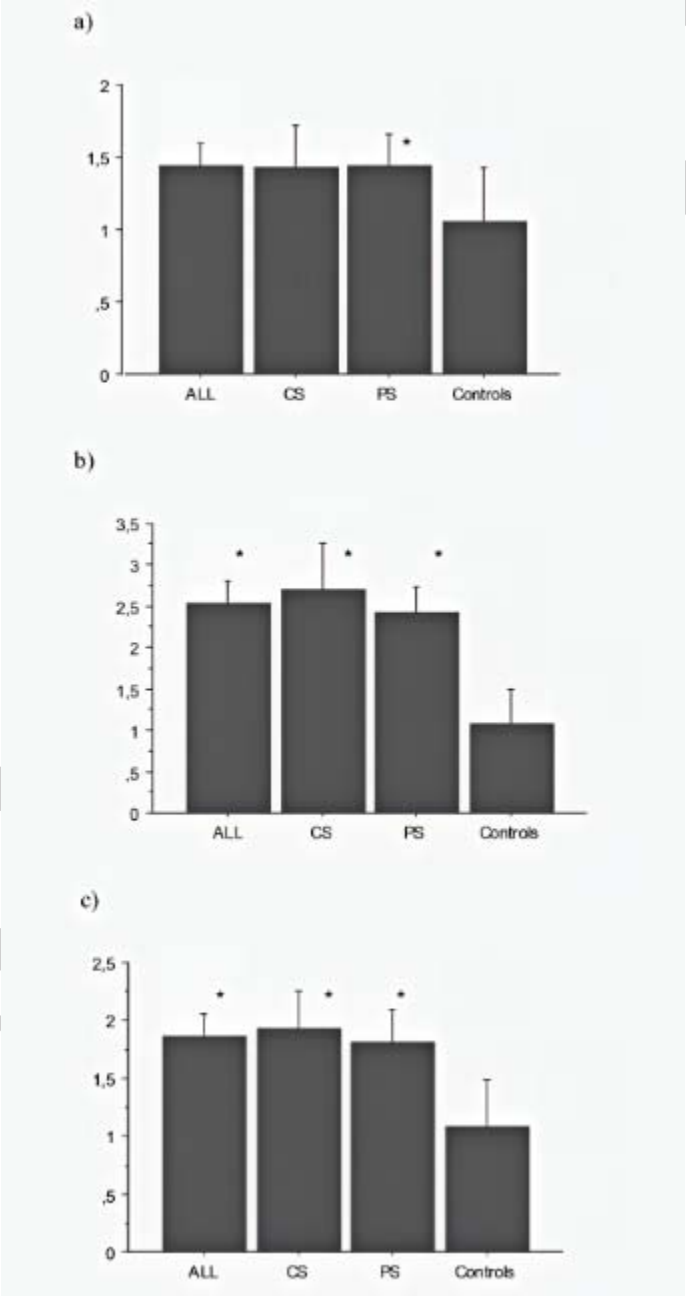

Figure 2. Liver RNA expression among all gallstone patients (ALL, n=32), patients with cholesterol stones (CS, n=15), patients with pigment stones (PS, n=17) and controls (n=6) for (a) *FXR*, (b) *ABCB4*, and (c) *ABCB11*. Data are fold changes (mean with 95% confidence interval) in relation to controls. *p < 0.05 vs controls **p<0.05 vs PS (Mann-Whitney U test).

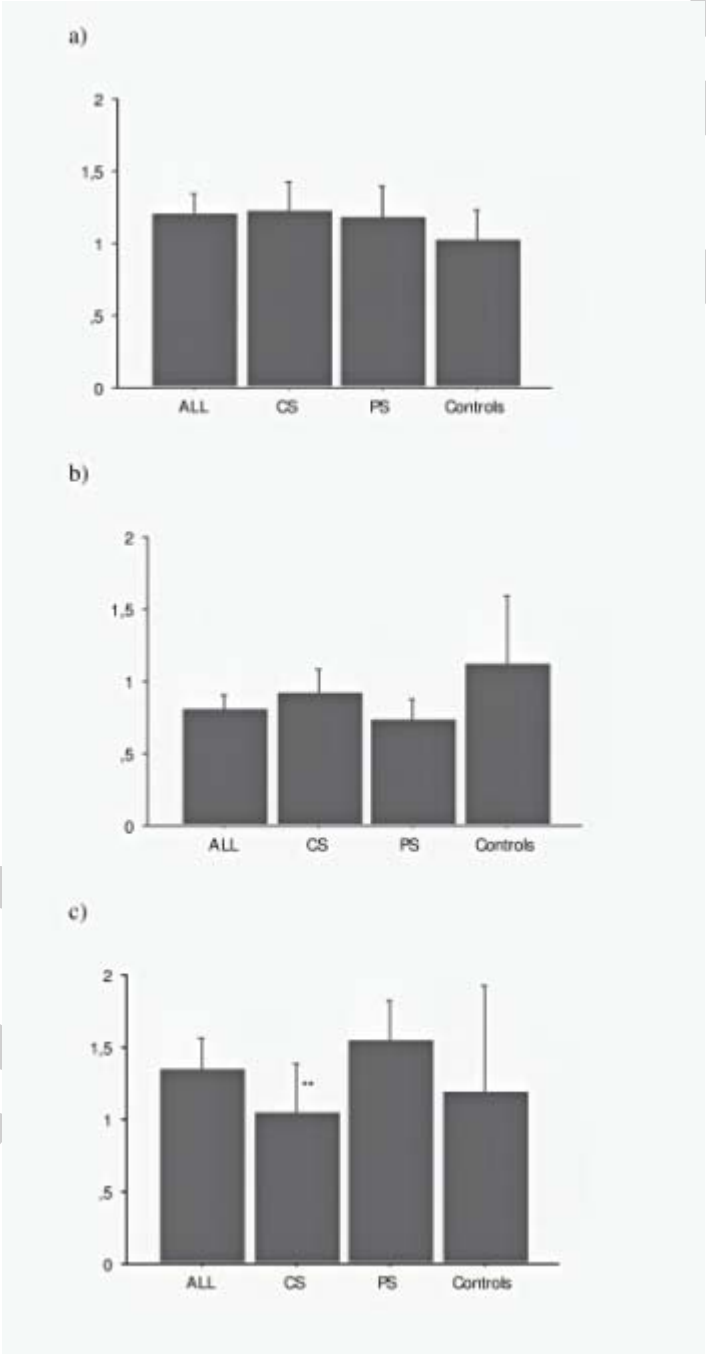

Table 1. Patient demographics, serum lipids and liver biochemistries and liver and gallbladder histology in patients with cholesterol and pigments stones.

	All patients	Cholesterol stone	Pigment stones	
	(n=32)	(n=15)	(n=17)	p*
Age, y	13 (10–16)	15 (12–16)	12 (8–15)	0.08
Females, n (%)	19 (59)	10 (67)	9 (53)	0.49
Iso-BMI, kg/m^2	23 (19–27)	27 (19–31)	21 (19 – 23)	**0.03**
Cholesterol, mmol/l	3.6 (3.2–4.2)	4.0 (3.5–4.5)	2.9 (2.5–3.7)	**0.04**
HDL, mmol/l	1.3 (1.0–1.5)	1.3 (1.3–1.7)	1.2 (0.8–1.3)	0.96
LDL, mmol/l	2.0 (1.6–2.5)	2.3 (1.9–2.6)	1.6 (1.3–2.2)	**0.02**
Triglycerides, mmol/l	1.0 (0.6–1.3)	0.6 (0.4–1.3)	1.0 (0.8–1.0)	0.85
Alanine aminotransferase, U/l	28 (23–31)	24 (22–30)	28 (25–30)	0.32
Aspartate aminotransferase, U/l	19 (16–30)	18 (16–19)	18(13–23)	0.29
Alkaline phosphatase, U/l	194 (96–251)	228 (75–269)	205 (137–247)	0.71
Bilirubin (µmol/l)	8.0 (5.0–9.5)	5.0 (5.0–7.0)	9.0 (7.0–11)	0.12
Glutamyl transferase, U/l	18 (13–28	18 (13–22)	24 (13–27)	0.21
Bile acids, µmol/l	5.0 (3.2–8.5)	3.9 (2.7–12)	4.1 (2.3–7.2)	0.40
Prealbumin, mg/l	216 (189–257)	232 (199–242)	205 (169–268)	0.39
Predisposing condition, n (%)	14 (44)	5 (33)	9 (53)	0.31
Liver histology				
Abnormal, n (%)	12 (38)	6 (40)	6 (35)	0.99
Steatosis	7 (22)	5	2	
Steatohepatitis	1 (3)	0	1	
Fibrosis	1 (3)	1	1	
Intracellular cholestasis	2 (6)	0	2	
Portal inflammation	1(3)	0	1	
Gallbladder histology				
Abnormal, n (%)	27 (84)	15 (100)	12 (71)	0.05
Chronic cholecystitis	25 (78)	14	11	
Acute cholecystitis	1(3)	0	1	
Cholesterolosis	1 (3)	1	0	

Data are medians (interquartile range). *p-values refer to Mann Whitney U test between patients with cholesterol and pigment stones.

Table 2. Comparison of serum cholesterol and non-cholesterol sterols between patients with cholesterol or pigment stones and controls.

	Cholesterol stones (n=1	Pigment stones (n=1	Controls (n=82)	p*
Cholesterol	159 (142 – 166) [b]	134 (104-167)	140 (124–162)	0.07
Precursors				
Cholestenol	23 (16–28)[a,b]	12 (10–20)	15 (11–18)	0.02
Lathosterol	163 (116–198)[a,b]	82 (59–98)	80 (57–106)	0.002
Desmosterol	96 (75–121)	88 (71–111)	86 (75–94)	0.42
Cholestanol	125 (106–149)[a,b]	178 (169–2241)[a]	162 (143–185)	<0.0001
Plant sterols				
Campesterol	152 (102–194)[a,b]	286 (218–437)	304 (220–356)	0.0001
Sitosterol	61 (59–117)[a,b]	176 (120–210)	163 (134–208)	<0.0001
Avenasterol	34 (26–38)[a]	40 (33–52)	46 (36–53)	0.01
Stigmasterol	11 (7–17)[a]	17 (12–19)[a]	27 (23–34)	<0.0001
Lathosterol/Sitosterol	2.0 (0.9–2.6)[a,b]	0.5 (0.4 –0.7)	0.4 (0.3–0.8)	< 0.001
Lathosterol/Cholestanol	1.3 (0.9 – 1.7)[a,b]	0.4 (0.3 – 0.5)	0.5 (0.3 – 0.7)	0.001
Campesterol/Cholestanol	1.2 (0.8 – 1.5)[a]	1.4 (1.3 – 2.1)	1.8 (1.4 – 2.1)	0.003

Data are medians (interquartile range). Cholesterol mg/dl, non-cholesterol sterols 100 x µg/mg of cholesterol. *p-values refer to Kruskall Wallis test among the three groups. [a]$p < 0.05$ compared to controls, [b]$p < 0.05$ compared to PS patients by subsequent Mann Whitney U test.